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RAT LIVER CYSTEINE SULFINATE DECARBOXYLASE: PURIFICATION, NEW APPRAISAL OF THE MOLECULAR WEIGHT AND DETERMINATION OF CATALYTIC PROPERTIES*

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Summary

Rat liver cysteine sulfinate decarboxylase (L-cysteine sulfinate carboxy-lase) was purified approximatively 500-fold. By cellulose acetate and polyacrylamide gel electrophoresis or by analytical ultracentrifugation, the purified enzyme appears to be nearly homogeneous. The Stokes radius (3.4 nm) and sedimentation coefficient (6.5 *S*) were determined. The molecular weight, calculated and experimentally estimated is around 100 000 and the enzyme is constituted of two identical subunits whose molecular weights are 55 000. The role of pyridoxal phosphate as coenzyme was demonstrated and the requirement for free sulfhydryl groups for activity was studied. The ability of native pure cysteine sulfinate decarboxylase to also decarboxylate cysteate was stressed: therefore, we concluded that in rat liver a single protein catalyzed both reactions, although only the decarboxylation of cysteine sulfinate is of physiological interest.

Introduction

It has been announced [1] that rat liver cysteine sulfinate decarboxylase (L-cysteine sulfinate carboxy-lyase) has been purified to near homogeneity and that the physical, chemical and catalytic properties of the enzyme have been determined. Several of the results obtained were in agreement with those of Lin et al., [2] however, others were not in keeping with their observations, mainly as regards the molecular weight and the substrate specificity. These discrepancies prompted us to thoroughly investigate these two points. This report deals

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with the method of purification set up and describes the properties of the enzyme, drawn from results of experiments performed with pure or partially purified enzyme preparations. In addition, in order to clarify the present knowledge about this enzyme, possibilities responsible for discrepancies between the observations of Lin et al. [2] and ours are suggested.

Materials and Methods

Animals

Adult male Wistar rats were obtained from the "Centre de Sélection des animaux de laboratoire du C.N.R.S." and fed a commercial diet (UAR-Entretien).

Chemicals

L-cysteine sulfinic acid was purchased from Cyclo Chemical (Los Angeles, U.S.A.); L-cysteic acid and dithiothreitol from Calbiochem (Los Angeles, U.S.A.); pyridoxal 5′-phosphate (Pyridoxal 5′-*P*) from Koch-Light (Colnbrook, U.K.); Amberlite CG-50 from Rohm and Haas (Philadelphia, U.S.A.); DEAE-cellulose DE-32 from Whatman (Maidstone, U.K.); Sephadex G-25 and G-100 from Pharmacia Fine Chemicals (Uppsala, Sweden).

Buffers

Only phosphate buffers were used; Na_2HPO_4/KH_2PO_4 generally and Na_2HPO_4,/NaH_2PO_4 for acrylamide gel electrophoresis. EDTA, pyridoxal 5′-*P* or dithiothreitol when added, were at 0.1 mM concentration.

Enzyme assay.

Decarboxylations were measured manometrically, through evolution of CO_2, under a nitrogen atmosphere in the conditions previously reported [1].

Proteins

Proteins were assayed by the method of Lowry et al. [3] using bovine serum albumin as standard. Results are expressed in μl CO_2 released per h, or μmol CO_2 per min, or units of enzyme, defined as the amount of enzyme which produced 1 μl CO_2 per min under the standard assay conditions.

Analytical procedure

Three methods were used: cellulose acetate electrophoresis (in 50 mM buffer (pH 7.8) with 185 V per stripe); polyacrylamide disc electrophoresis (1 ml of 7.5% gel in 50 mM buffer (pH 8.1) in a Quickfitt apparatus; the current was 10 mA per gel and the proteins were colored with Coomassie blue "R"); ultracentrifugation in an "analytical Spinco ultracentrifuge" equipped with a "Schlieren optical system" (using a preparation of enzyme containing 4 mg protein/ml, in 20 mM buffer (pH 6.8) added with pyridoxal 5′-*P*, dithiothreitol and EDTA), or in a "MSE-Centriscan 75 ultracentrifuge" equipped with a photoelectric scanning system at 280 nm (using a sample containing 1 mg enzyme/ml in the same buffer).

Results

Purification procedure

All operations were performed at 4°C. Fractions decarboxylating cysteine sulfinate were referred to as active fractions.

Step 1: Extraction. Rats were killed by decapitation and the livers were perfused with cooled 0.9% NaCl, then excised, weighed, and a 33% w/v liver suspension was prepared in cooled 0.25 M sucrose containing 0.1 mM EDTA, with an Ultra-Turrax homogenizer. The supernatant fluid "S_1" was isolated by centrifuging at 78 000 × *g* for 60 min in a Spinco ultracentrifuge.

Step 2: Heat treatment. S_1 was added with 0.1 mM pyridoxal 5′-*P* and heated at 55°C for 5 min, then cooled: the precipitate was discarded and activity was present in supernatant S_2.

Step 3: First $(NH_4)_2SO_4$ fractionation. S_2 was added with solid ammonium sulfate until 50% saturation. The precipitate was dissolved in 20 mM buffer (pH 6.8) containing EDTA, pyridoxal 5′-*P* and dithiothreitol (buffer I). Desalting was performed on a Sephadex G-25 column (100 × 4 cm) pre-equilibrated with 20 mM buffer (pH 5.65) containing EDTA (buffer II).

Step 4: Amberlite CG-50 column chromatography. The desalted active fraction was applied onto an amberlite CG-50 column (30 × 2.8 cm) preequilibrated with 0.2 M buffer (pH 6.5) (buffer III) containing EDTA, pyridoxal 5′-*P* and dithiothreitol, and the column was washed with the same buffer. The absorption at 280 nm of the collected fractions was monitored and an elution pattern was recorded in an "Elugraphe" or "DO-graphe" (Seive manufacturers); active fractions were detected and pooled; the enzyme passed straight through the column

Step 5: Second $(NH_4)_2SO_4$ fractionation. The fractions precipitated at 30—40%, and also at 40—60% ammonium sulfate saturation, were collected, respectively dissolved in 10 mM buffer (pH 7.4) containing EDTA, pyridoxal 5′-*P* and dithiothreitol (buffer IV) and dialysed overnight against three changes of the same buffer. The first sample, which contains a part of the enzyme associated with impurities, could be used for preliminary studies; the second one, which gave a preparation of higher purity and activity, was used for the following steps.

Step 6: DEAE-cellulose column chromatography. This active fraction was applied onto a DEAE-cellulose column (25 × 1.8 cm) pre-equilibrated with buffer IV. Elution was performed stepwise using buffers of increasing concentration, from 10 to 50 mM (Fig. 1). Active fractions were present in the second peak; they were pooled, added with 0.1 mM EDTA, pyridoxal 5′-*P* and dithiothreitol and concentrated to a maximum of 6—7 ml by ultrafiltration in a Amicon Diaflo apparatus using a PM 30 filter.

Step 7: Sephadex G-100 gel filtration. The active solution was applied onto a Sephadex G-100 column (100 × 2.6 cm) pre-equilibrated with 20 mM buffer (pH 6.8) containing EDTA, pyridoxal 5′-*P* and dithiothreitol (buffer V). The elution was performed with the same buffer, the flow-rate being 12 ml/h. Amongst the active fractions, those having high activity and also giving a single band on cellulose acetate electrophoresis were pooled and concentrated by ultrafiltration (Fig. 2). As a representative example, data obtained in one ap-

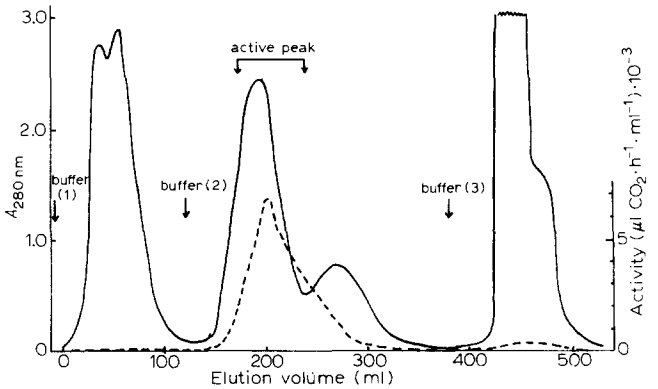


Fig. 1. Chromatography on DEAE-cellulose (DE-32). 362 mg of protein, obtained after the second $(NH_4)_2\ SO_4$ fractionation (40—50% satn.), desalted and equilibrated in 10 mM buffer (pH 7.4), containing EDTA, PLP, dithiothreitol, was applied onto the column (25 × 1.8 cm) equilibrated in the same buffer. Elution was performed stepwise, using buffers (pH 7.4) of increasing concentrations with 0.1 mM EDTA: (1) = 10 mM, (2) = mM, (3) = 50 mM buffers. Fractions of 2 ml were collected and assayed for absorbance at 280 nm (———) and enzyme activity (- - - - -). (↓———↓) denotes the fractions that were pooled and concentrated for chromatography on Sephadex G-100.

plication of the procedure are given in Table I. The method was described for 300-g liver batches, however, it can be applied, without modifying the three last steps, for double liver weight; in this case, steps 1, 2, 3 and 4 were performed twice, and this was done in the given example. In earlier experiments a CM-cellulose chromatography was included in the purification procedure but later it has been observed that this step could be conveniently replaced by Sephadex G-100 filtration.

Homogeneity

Purified cysteine sulfinate decarboxylase migrates as a single band during cellulose acetate electrophoresis; in acrylamide gel electrophoresis a major band

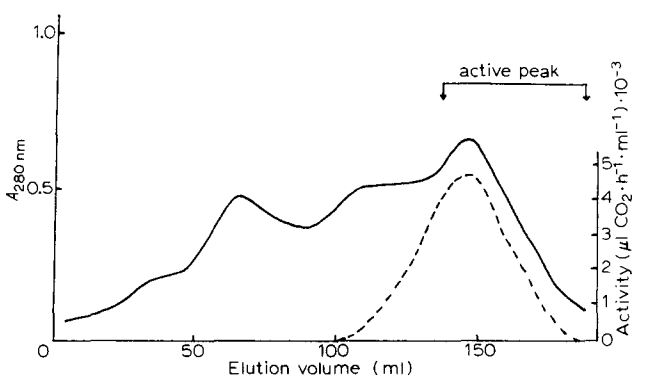


Fig. 2. Gel filtration on Sephadex G-100. 7 ml of concentrated DEAE-cellulose purified enzyme containing 58 mg of protein were applied into the column (100 × 2.6 cm). Elution was performed with 20 mM buffer (pH 6.8) containing 0.1 mM EDTA, with a flow-rate of 12 ml/h. Fractions of 4 ml were collected and assayed for absorbance at 280 nm (———) and enzyme activity (- - - - -). (↓———↓) represents the Sephadex G-100 purified enzyme.

TABLE I

PURIFICATION OF RAT LIVER CYSTEINE SULFINATE DECARBOXYLASE

617 g of fresh rat liver (52 animals) were used, treated in two separate batches for the four first steps, as described in the text.

Step	Total protein (mg)	Total activity (units)	Specific activity (units·mg protein^{-1})	Yield	Purification
Whole homogenate	97900	850	0.0087	100	1
Supernatant S_1	37250	757	0.0203	89	2.34
Supernatant S_2	14350	531	0.0371	62.5	4.26
$(NH_4)_2SO_4$ fraction (50% satn)	3130	484	0.1545	56.6	17.8
Amberlite pool	1450	432	0.2980	50.7	33.1
$(NH_4)_2SO_4$ fraction (40—50% satn)	362	250	0.690	29.4	79.4
DEAE-cellulose pool	58	150.5	2.60	17.6	299
Sephadex G-100 pool (concentrated)	12.2	51.3	4.21	6.05	485

was associated with a trace of impurity or denaturated enzyme; run in an analytical ultracentrifuge, the purified enzyme (4 mg protein/ml) sediments essentially as one peak which represents 85—97% of the total proteins (Fig. 3).

Stability

Purified enzyme solutions with EDTA, pyridoxal 5'-*P* and dithiothreitol added were stored in lots of 3—5 mg of proteins and frozen at —20° C. When thawed, a lot was used only once. Under those conditions the loss of activity was about 25—30% between 2 weeks and 2 months.

Physical properties

Absorption spectrum

The freshly prepared purified enzyme dialyzed against a 20 mM buffer (pH 6.8) containing EDTA, pyridoxal 5'-*P* and dithiothreitol exhibited a characteristic ultraviolet spectrum (Fig. 4). Besides the peak at 280 nm due to aromatic amino acid residues, two smaller ones are seen at 335 nm and 435 nm. The second gave evidence for a Schiff's base between pyridoxal 5'-*P* and protein; the first was probably due to dithiothreitol associated with the Schiff's base, as already observed for other pyridoxal 5'-*P* proteins in the presence of reducing agents [4,5].

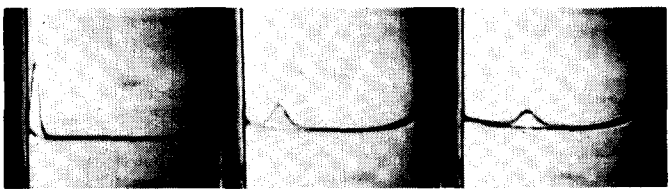
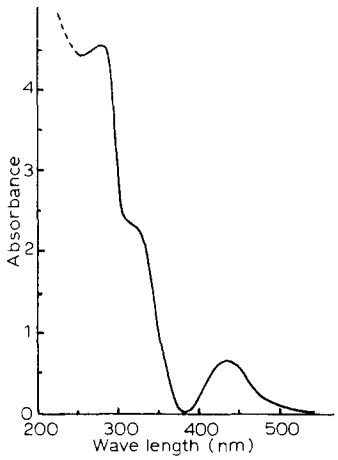


Fig. 3. Analytical ultracentrifugation of Sephadex G-100 purified enzyme, containing 4 mg of protein per ml, in 20 mM buffer (pH 6.8) added with EDTA, PLP and dithiothreitol. Photographs were taken at 3, 43 and 83 min after the full speed (59,780 rev/min) had been attained. Temperatures was 4° 30'C.



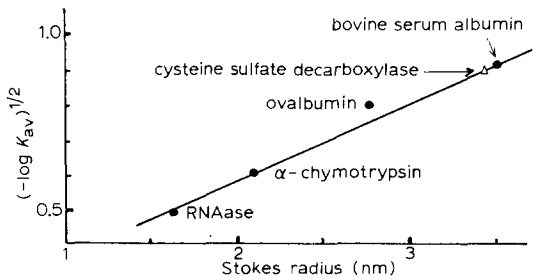


Fig. 4. Absorption spectrum of Sephadex G-100 purified cysteine sulfinate decarboxylase (4 mg/ml 20 mM buffer (pH 6.8) added with EDTA, PLP and dithiothreitol), recorded in a Leres-Spila spectrophotometer.

Fig. 5. Determination of Stokes radius of cysteine sulfinate decarboxylase by gel filtration on Sephadex G-100. The Sephadex G-100 column (115 × 1.5 cm), with a total volume (V_t) of 204.5 ml, was calibrated with 1 ml solutions containing the standard proteins, and blue dextran-2000 for void volume (V_o) determination. Elution was performed with a flow rate of 12 ml/h. The elution volumes (V_e) for standard proteins and for enzyme were determined and $(-\log K_{av})^{\frac{1}{2}}$ was plotted versus the known Stokes radius. $K_{av} = (Ve - Vo)/(V_t - V_o)$.

Isoelectric point

This was estimated as the pH value where no migration of the protein occurred in cellulose acetate electrophoresis; this value was 5.1 ± 0.1.

Molecular parameters

Stokes radius was measured by means of Sephadex G-100 gel filtration, carried out according to Laurent and Killander [6] and Siegel and Monty [7]; using a partially purified enzyme solution, the active fraction was detected by its enzymic activity. The Stokes radius was estimated to be 3.4 nm (Fig. 5).

Sedimentation coefficient. Two methods were used for the determination of the sedimentation coefficient $s_{20,w}$. By sucrose density gradient centrifugation, according to Martin and Ames [8], a $s_{20,w}$ value of 6.4 S was obtained (Fig. 6); by analytical ultracentrifugation at 20° C or at 4° C of a sample of pure enzyme containing 1 mg protein per ml, we measured a $s_{20,w}$ of 6.5 S.

Molecular weight. Calculation of the molecular weight using the last two parameters was carried out from the classical equation $M = 6\pi\eta Na\, s_l/(1 - v\rho)$ where M is the molecular weight, a is the Stokes radius, s is the sedimentation coefficient, η is the viscosity of the medium, ρ is the density of the medium, N is Avogadro's number and v is the partial specific volume of the protein assumed to be 0.735 $cm^3 \cdot g^{-1}$. Such a calculation gave a molecular weight of 96 000. Experimental determination was performed by polyacrylamide gel disc electrophoresis of pure native enzyme using various gel concentrations according to Hedrick and Smith [9]. A molecular weight value of 105 000 ± 4000 was obtained (Fig. 7), which is in good agreement with the calculated value.

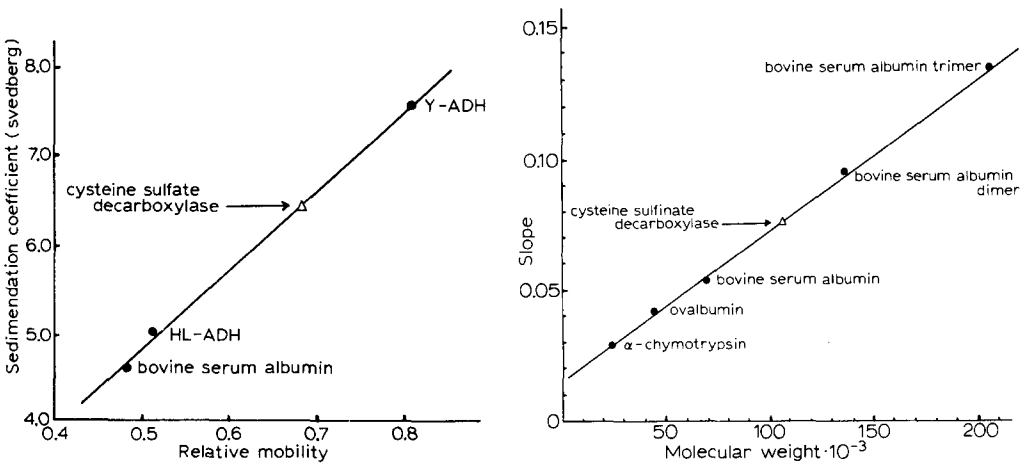


Fig. 6. Determination of sedimentation coefficient of cysteine sulfinate decarboxylase by density gradient centrifugation. 5—20% (w/v) sucrose gradients in 20 mM buffer (pH 6.8) containing EDTA, PLP and dithiothreitol were prepared. Standard proteins (Yeast- and Horse Liver-alcohol dehydrogenase = Y-ADH and HL-ADH), or cysteine sulfinate decarboxylase solutions, i.e. 2—4 mg of each in 0.1 ml, were applied onto the plane of the 6-ml gradient tubes which were centrifuged in a MSE swinging rotor at 52 000 rev./min for 16 h, at 4°C. The sedimentation coefficient of the standard was plotted versus the relative mobility.

Fig. 7. Determination of molecular weight of cysteine sulfinate decarboxylase by acrylamide gel electrophoresis. 5 gel concentrations (5, 7, 9, 11, and 13%) in 50 mM buffer (pH 8.1) were used. 20—30 μg of protein (standard or pure enzyme) with bromophenol blue in a maximum volume of 30 μl were applied onto each gel. Experiments were performed according to Hedrick and Smith [9] with 10 mA per tube for 1 h: negative slopes for the standards and for the enzyme were plotted versus the known molecular weight.

Subunits

Dodecylsulfate disc electrophoresis on polyacrylamide gel was carried out according to the method of Fairbanks et al. [10] in 1% containing 0.1 M 2-mercaptoethanol. Treated at 100°C with 1% sodium dodecylsulfate containing 0.1 M 2-mercaptoethanol, the purified enzyme gave one band. From Fig. 8, the molecular weight was estimated to be 55 000 ± 2500. This suggests that the native enzyme, whose molecular weight is around 100 000, is constituted of two subunits having the same molecular weight. Moreover, when treated with 7 M urea, the purified enzyme lost its activity, and, on polyacrylamide gel electrophoresis in 7 M urea, gave only one band. This indicated that the two subunits of the same molecular weight have also the same electrical charge, and thence are presumably identical.

Role of pyridoxal phosphate

Addition of pyridoxal 5′-*P* to freshly prepared rat liver homogenates and to semi-purified preparations did not significantly increase the rate of cysteine sulfinate decarboxylation. However, the enzyme was considered as a pyridoxal 5′-*P* enzyme due to its high susceptibility to pyridoxine deficiency of the animal [11,12], and also according to results obtained with isonicotinyl hydrazide [13]. On the other hand we previously reported that pyridoxal 5′-*P* protected the enzyme against denaturation by heat or urea and that neither

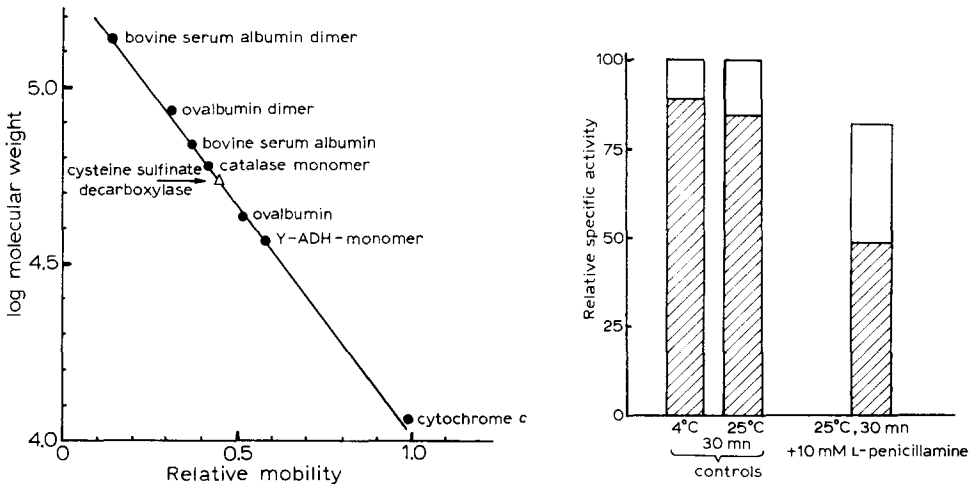


Fig. 8. Determination of the molecular weight of cysteine sulfinate decarboxylase using 1% dodecylsulfate acrylamide gel electrophoresis, according to Fairbanks et al. [10]. 10—20 μg of standard proteins and pure enzyme treated with 1% dodecylsulfate were used and the electrophoresis was performed at pH 8.1 with 7.5 mA per tube for 1½ h. The mobility was plotted versus the log molecular weight of the standards. (The standards were the same as described in the preceding figure with the addition of catalase monomer).

Fig. 9. Effect of incubation cysteine sulfinate decarboxylase with 10 mM L-Penicillamine on enzyme activity. The enzyme solutions were kept at 4° C or 25° C for 30 min without reagent (controls) or at 25° C for 30 min with 10 mM L-Penicillamine, and then run on Sephadex G-25 columns to deplete the L-Penicillamine. Cysteine sulfinate decarboxylation activities were measured with (▭) or without (▨) 0.1 mM PLP in the assays.

pyridoxal nor pyridoxamine 5′-*P* exhibited this property [14]. This observation suggests a role of pyridoxal 5′-*P* in holding the native protein conformation. Furthermore, the ultraviolet spectrum of the pure enzyme gave evidence that cysteine sulfinate decarboxylase is a pyridoxal 5′-*P* enzyme (Fig. 4). Therefore, some experiments were set up to clarify the role of pyridoxal 5′-*P*.

Effect of sodium borohydride: Treatment of the enzyme with 0.1 M borohydride for 1 h at 4° C produced an important loss of activity since the residual activity was less than 10% of that of the untreated control. No reactivation was afforded by the addition of pyridoxal 5′-*P* in the assay. These observations strongly suggest that pyridoxal 5′-*P* plays a role in enzyme catalysis.

Effect of reagents of pyridoxal 5′-P: Noradrenaline and penicillamine [15,16] complex pyridoxal 5′-*P*, forming, respectively, and oxazolidine or a thiazolidine ring with the aldehyde group of pyridoxal 5′-*P*. We observed that the two reagents had similar effects. As an example, Fig. 9 presents the results obtained with penicillamine which is the most active. An enzyme solution, deprived of pyridoxal 5′-*P* by a quick run on a Sephadex G-25 column, incubated at 4° C or 25° C for 30 min, exhibited a loss of activity which could be completely reversed by the addition of 0.1 mM pyridoxal 5′-*P* in the assay. When 10 mM penicillamine was added prior to incubation, the inactivation observed was far much important and a good deal of the activity was recovered by the addition of 0.1 mM pyridoxal 5′-*P* in the assay. Therefore, it appears that penicillamine trapped the pyridoxal 5′-*P* bound to the enzyme, thus giving an apoenzyme which still has the capacity to bind pyridoxal 5′-*P* and thence to

recover at least a part of the activity. These results confirm that cysteine sulfinate decarboxylase is a pyridoxal 5'-*P* enzyme; the observation that the reactivation of the apoenzyme by pyridoxal 5'-*P* was not entire, strongly suggests that apoenzyme itself is very unstable in vitro. Assuming that a similar unstability also occurs in vivo may explain the peculiar susceptibility of the enzyme to pyridoxine deficiency.

Substrate specificity

As previously pointed out [1] the problem of substrate specificity needs clarification. Does a single enzyme catalyse, in rat liver, the decarboxylation of both cysteine sulfinate and cysteic acid which is the generally accepted concept? Or does a decarboxylase specific for cysteine sulfinate exist, as reported [2]? In contradiction to the last claim, we had reported [1] that in every enzyme solution checked and at every step of the purification, including the pure enzyme, the ratio of the rates of decarboxylation of cysteine sulfinate and cysteic acid was constant.

Therefore, measures of kinetic constants were performed whose results did confirm the previously reported values for Michaelis constants [17,2]: for cysteine sulfinate, K_M was 0.17 mM and the specific molecular activity was 6.1 · 10^2 mol cysteine sulfinate · mn^{-1} · mol $enzyme^{-1}$; for cysteic acid, the K_M was 0.66 mM and the specific molecular activity was 1.06 · 10^2 mol cysteic acid · mn^{-1} · mol $enzyme^{-1}$.

Moreover, the same pH dependence was observed (Fig. 10) for the two reactions. Susceptibility to heat, examined at 57°C and 59°C which are the

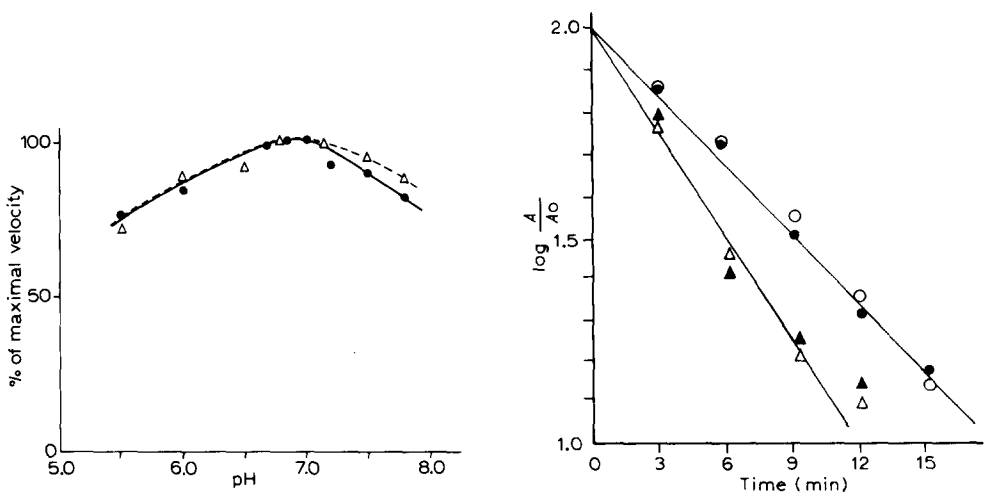


Fig. 10. Influence of the pH of the reaction on the decarboxylation rates of cysteine sulfinate (●——●) or cysteic acid (△- - - - -△). For each substrate, the activities were expressed as a percentage of the velocity at optimum pH.

Fig. 11. Thermal sensitivities of cysteine sulfinate and cysteic acid decarboxylations. *A* is the decarboxylation rate at *t* time and A_0 at zero time. log A/A_0 were plotted versus the time of incubation at 57°C (● = cysteine sulfinate and ○ = cysteic acid decarboxylation) and at 59°C (▲ = cysteine sulfinate and △ - cysteic acid decarboxylation). Slopes, which represent the constants of the inactivation rates for cysteine sulfinate and cysteic acid are 0.058 · min^{-1} at 57°C and 0.080 · min^{-1} at 59°C.

denaturing temperatures, was also the same (Fig. 11). These results, on the one hand, strongly confirm that a single protein is responsible for the decarboxylation of both substrates; on the other hand, associated with the observation [1] that no addition of the two decarboxylation rates occurred when cysteine sulfinate and cysteic acid were present at the same time in the assay, they favor the assumption that a single active site catalyzes the two reactions.

Role of sulfhydryl groups

The requirement for sulfhydryl reducing agents in the assay to produce maximal activity has been known for years [18,19,2]; for instance, in all our experiments dithiothreitol was routinely added, and we observed that the efficiency of 2-mercaptoethanol or reduced glutathione was similar to that of dithiothreitol.

In agreement with earlier results [18,2], we observed an important inhibition of the enzyme activity by *p*-chloromercuribenzoate. However, a quantitative study of the effect of this reagent on the enzyme was difficult, due to the great susceptibility of the SH groups necessary for the activity. As already described [18] the rate of cysteine sulfinate decarboxylation of a partially purified preparation was constant, but only after a lag period during which CO_2 production was lower, and this lag period was completely abolished when a reducing agent was present. Such a feature did not appear when cysteic acid decarboxylation was measured: in that case, a constant rate of CO_2 production occurred immediately, even without reducing agent, although the rate of decarboxylation was higher in its presence. The lag period observed for cysteine sulfinate decarboxylation had been considered as resulting from the reduction, by the substrate itself, of the SH groups of the enzyme necessary for maximal activity. Of course, nothing similar can occur with cysteic acid. Our feeling is that, although no chemical model is available, such an interpretation is the most plausible. On the other hand we observed that the product of the reaction hypotaurine, which did not inhibit cysteine sulfinate decarboxylation was

TABLE II

EFFECT OF DITHIOTHREITOL ON THE CYSTEINE SULFINATE AND CYSTEIC ACID DECARBOXYLATION RATES

A partially purified enzyme solution, stocked without dithiothreitol, was used. Activities were measured in the presence of increasing concentrations of dithiothreitol in the assay.

Dithiothreitol concentration in the assay (*M*)	Decarboxylation rates		*A/B*
	Cysteine sulfinate substrate (*A*): % of max. activity	Cysteic acid substrate (*B*): % of max. activity	
0	69.5*	13.2	34
10^{-6}	65*	13.2	32
10^{-5}	70.5*	13.2	34.5
$5 \cdot 10^{-5}$	85**	52	10.8
10^{-4}	85**	84	6.7
10^{-3}	100**	100	6.5

* The cysteine sulfinate decarboxylation rate as measured after a lag period.

** The cysteine sulfinate decarboxylation rate obtained at the beginning of the reaction.

unable to "reactivate" SH groups, in other words to act like cysteine sulfinate. It ensues that the carboxylic group of cysteine sulfinate is of importance in the process of "reactivation". Therefore, we paid attention to another aspect to the problem: the influence of the status of the enzyme preparation, as far as degree of reduction is concerned, on the ratio of the decarboxylation rates of the two substrates.

Table II reports the change of the ratio of the decarboxylation rates of the two substrates when the concentration of dithiothreitol in the assay was increased. Clearly, the ratio was very high (A/B = 34) without reducing agent and it approached the currently observed values (5—6) when 1 mM dithiothreitol was added. Thus it is obvious that no comparison of the two decarboxylating activities of an enzyme preparation is possible in the absence of suitable reducing conditions which means the presence of a reducing agent and also the absence of oxygen in the assays [1].

Discussion and Conclusion

From the results described in this report and from those already published, the present knowledge about rat liver cysteine sulfinate decarboxylase may be drawn. The protein is an acid one (isoelectric point = 5.1), and is constituted of two presumably identical subunits of 55 000 daltons; the molecular weight of the native enzyme is around 100 000. The previously reported value (66 000) estimated by Sephadex G-100 or Bio Gel P-150 gel filtration [1,2] cannot be taken into account. Indeed features of the enzyme, like sedimentation coefficient (6.5 S) and Stokes radius (3.4 nm), were determined which, when taken altogether, were inconsistent with this value, but were in agreement with the molecular weight experimentally determined by other methods. Therefore, we concluded that gel filtration was an irrelevant method for estimation of the molecular weight of cysteine sulfinate decarboxylase, as previously observed for other proteins [20]. Indeed it seems that the behavior of a protein on such a gel column depends upon its molecular shape rather than upon its molecular weight.

Cysteine sulfinate decarboxylase is a pyridoxal 5′-P enzyme which is normally saturated with coenzyme in rat liver. When deprived of pyridoxal 5′-P the protein is peculiarly unstable. According to our results pyridoxal 5′-P interferes in the holding of the native conformation (protective effect against denaturation) and also in the catalysis (inactivating effect of sodium borohydride, noradrenaline and penicillamine); however at the moment it is not possible to state whether the same pyridoxal 5′-P molecule plays those different roles.

Free sulfhydryl groups required for full activity are presumably very fragile since the addition of SH reducing agents as well as measure of activity under a nitrogen atmosphere is a prerequisite in order to obtain maximal activity in vitro. We can assume that in vivo the high concentration of non-protein SH groups of rat liver [21] could keep the enzyme in the active form.

We observed that the highly purified preparation of enzyme does decarboxylate cysteine sulfinate and cysteic acid and furthermore that the ratio of activities is the same as that of a rat liver homogenate (A/B = 5—6). The affinity of the enzyme for cysteine sulfinate and the rate of decarboxylation of

this substrate are respectively higher than the same parameters for cysteic acid. These observations associated with the very low concentration of cysteic acid in the rat liver [22,23] strongly suggest that the effective reaction is the decarboxylation of cysteine sulfinate.

Finally we underlined that, when both activities were measured on a partially purified preparation whose SH groups are not kept in reduced state, a quite different ratio (A/B = 34) was obtained. Such a ratio could erroneously suggest that a separation of the two activities, in favor of cysteine sulfinate decarboxylation, was achieved in the purification. However, our interpretation is that, in that case, cysteine sulfinate through its reducing properties has the capacity to "reactivate" some of the oxidized SH groups of the enzyme, whereas cysteic acid is unable to act similarly.

Our conclusion is that in rat liver, a single protein catalyses the decarboxylation of both cysteine sulfinate and cysteic acid. The "normal" substrate is cysteine sulfinate which is a physiological intermediate product between cysteine and taurine. However, should the occasion arise that cysteic acid is present in appreciable amounts in rat liver (either pathologically formed or due to diet) cysteine sulfinate decarboxylase could decarboxylate it under physiological conditions.

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